



## Analysis of Secondary Metabolite Composition in n-Hexane Oil Extract from *Jathropa gossypifolia* Seeds Using Gas Chromatograph Mass Spectrometer (GC-MS) Method

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### ABSTRACT

With its tropical climate, Indonesia is a home to many *Jatropha gossypifolia* plants, which are often underutilized and considered as weeds by the public. This plant contains various active compounds such as alkaloids, tannins, saponins, terpenoids, lignans, phenolics, flavonoids, curcins, triterpenes, diterpenes, jatrophone, cyclogossin A, steroids, sugars, amino acids, proteins, coumarins, and fatty acids. This study aimed to analyze the secondary metabolite composition of n-hexane oil extracts from raw and roasted *J. gossypifolia* seeds using Gas Chromatograph Mass Spectrometer (GC-MS) methods. The extraction was carried out using n-hexane to obtain the oil from both raw and roasted seeds, and the compositions were analyzed using GC-MS. Chromatogram results showed 14 active peaks from the roasted seed extract and 27 peaks from the raw seed extract. For roasted seeds, the main components are ethyl 9,12-octadecadienoate (30.17%), (Z)6,(Z)9-pentadecadien-1-ol (19.59%), ethyl octadec-9-enoate (10.51%), ethyl ester of docosanoic acid (9.88%), hexadecanoic acid, ethyl ester (7.75%), and 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (4.14%). For raw seeds, the primary components are 9,12-octadecadienoic acid (16.08%), undecane, 5-ethyl- (9.43%), benzene, 1,3,5-trimethyl- (8.82%), 1-nonadecene (7.83%), 6,11-hexadecadien-1-ol (6.37%), 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (6.32%), and nonanamide (5.96%). The differences in secondary metabolite compositions between raw and roasted seeds indicate significant chemical changes during the roasting process. The main components found in the oil extracts of *J. gossypifolia* seeds have potential benefits for various applications. This research provides essential information on the potential utilization of *J. gossypifolia* seeds in health and industry fields.

**Keywords:** N-hexane extract, *Jatropha gossypifolia* seeds, Gas chromatograph mass spectrometer, Secondary metabolites.

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### Introduction

*J. gossypifolia* is a plant that grows in tropical regions such as Indonesia. This plant originates from North America and has spread to other regions.<sup>1</sup> In Indonesia, *J. gossypifolia* is distributed in Java, Madura, Sulawesi, and Timor, and it can adapt to various types of habitats, although it prefers dry lands, savannas, and is often considered a weed in grasslands.<sup>2</sup> On the island of Timor, *J. gossypifolia* is commonly found in lowlands to coastal areas and is generally used as a fence plant. This shrub can grow up to approximately 3 meters in height with young purple leaves covered with sticky hairs.<sup>3,4</sup> The use of the *J. gossypifolia* plant's parts as traditional and modern medicine have been widely practiced in Africa, Asia, and Latin America.<sup>5</sup> This plant is used to treat gastrointestinal, anticancer,<sup>6,7</sup> skin and brain diseases.

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According to the literature, *J. gossypifolia* contains active compounds such as alkaloids, tannins, saponins,<sup>10</sup> terpenoids,<sup>3</sup> lignans, phenolics, flavonoids, curcins, triterpenes, diterpenes, jatrophone, jatropholones A and B, jatrophatrione, apigenin, and cyclogossin A,<sup>11</sup> as well as steroids, fatty acids, sugars, amino acids, proteins, and coumarins.<sup>1</sup> The seed extract showed significant antifungal activity.<sup>12</sup> The phytochemical content found depends on the solvent and part of the plant, such as leaves, seeds, stems, roots, or sap.

Most *J. gossypifolia* seeds contain oil with the main components being fatty acids. The most commonly found component is heptadecanoic acid at 35.81%,<sup>13</sup> followed by palmitic and stearic acids,<sup>14</sup> which are saturated fatty acids, and oleic and linoleic acids,<sup>15</sup> which are unsaturated fatty acids,<sup>14,16</sup> hexadecanoic acid at 18.05%,<sup>17</sup> and methyl ester.<sup>18</sup> In East Nusa Tenggara, the identification and utilization of bioactive compounds in *Jatropha gossypifolia* have not yet been extensively studied. This study aims to analyze the secondary metabolite composition of n-hexane oil extract from raw and roasted *J. gossypifolia* seeds using the Gas Chromatograph Mass Spectrometer (GC-MS) method.

### Materials and Methods

#### Plant Collection and Identification

Seed samples were collected from a specific area called Lasiana in Kupang City, East Nusa Tenggara Province (GPS: 10.1429° S, 123.6707° E), Indonesia, from May to July 2024. The seeds were dried under the sun, covered with cloth until the kernels separated from the

shells. The seeds were then separated from their shells, resulting in 600 grams which were divided into two parts. A total of 300 grams were roasted at 100°C for 5 minutes, while the remaining 300 grams were left unroasted. Both samples were ground using a hand chopper blender into powder form. The whole plant was authenticated at the Biology Laboratory, Faculty of Science and Technology, Nusa Cendana University, Kupang, East Nusa Tenggara (reference number 920/UN 15.15.10/PP/2024).

#### Extraction

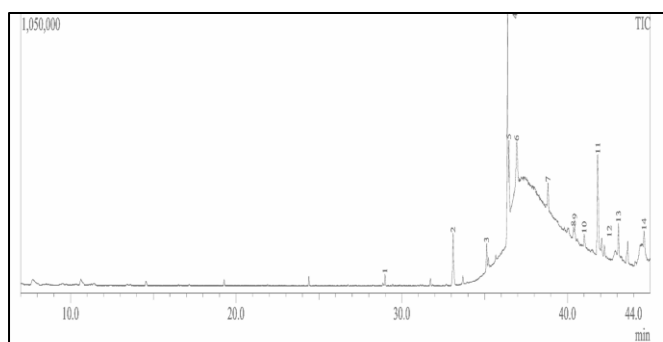
The extraction process was conducted using maceration with n-hexane Pro Analysis (Merck, Germany) following the method described by Hajrah et al.<sup>19</sup> Oils were extracted with a sample-to-solvent ratio of 1:3. Each sample, consisting of 300 grams of *Jatropha gossypifolia* powder, was dissolved in 900 mL of n-hexane<sup>20</sup> and allowed to sit for three days. The extract was then concentrated using a rotary evaporator until a brownish and clear yellow oil was obtained. Any remaining brown oil was separated from its sediment using filter paper.<sup>19</sup> The filtrate was collected in glass bottles and stored in a dark place. This extract was then subjected to qualitative tests (phytochemical screening) and the quantification of active compounds present in each extract.<sup>3</sup>

#### GC-MS Analysis

The extracts were further analyzed for their chemical content using a GC-MS QP-2010S (Shimadzu, Japan) instrument, following the method outlined by Senou et al.<sup>21</sup> The chromatographic instrument had the following specifications: column oven temperature set at 70.0°C, injection temperature at 310.0°C, injection mode was splitless, sampling time was 1.00 minute, flow control mode was pressure with a setting of 30.0 kPa, total flow rate was 55.8 mL/min, column flow rate was 0.65 mL/min, linear velocity was 29.6 cm/sec, purge flow was 3.0 mL/min, split ratio was set at 80.0, high pressure injection was turned off, and carrier gas saver was off. The separation results were identified using an MS detector, and the mass spectra were compared against the WILEY 7 database. The relative percentages of the oil components were calculated from the GC peak areas.<sup>22,23</sup>

## Results and Discussion

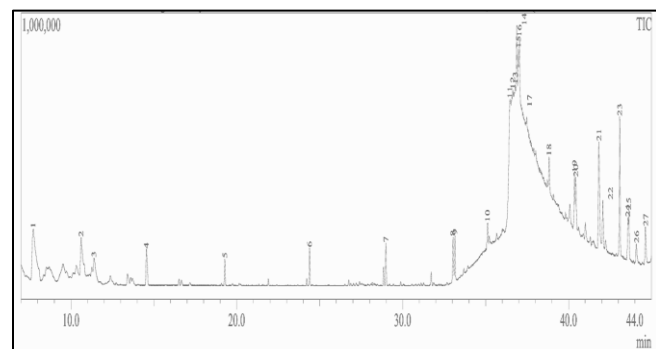
Mass spectrum analysis revealed a variety of metabolites, identified by comparison with the WILEY 7 library database. The chromatogram of the n-hexane extract from roasted *Jatropha gossypifolia* seeds exhibited 14 peaks (Figure 1), whereas the extract from raw seeds showed 27 peaks (Figure 2), with the corresponding active compounds identified for each peak (Table 1 and Table 2).



**Figure 1:** Chromatogram of roasted *J. gossypifolia* seed oil extract

Table 1 and Figure 1 present the percentage area of the major bioactive compounds in the roasted seed extract, including ethyl 9,12-octadecadienoate (linoleic acid, ethyl ester) at 30.17%, (Z)6,(Z)9-pentadecadien-1-ol at 19.59%, ethyl octadec-9-enoate at 10.51%, docosanoic acid, ethyl ester at 9.88%, hexadecanoic acid, ethyl ester (CAS: Ethyl palmitate) at 7.75%, and 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS: Bis(2-ethylhexyl) phthalate) at 4.14%. Meanwhile, Table 2 and Figure 2 show the dominant compounds in the

raw seed extract, which include 9,12-octadecadienoic acid (Z,Z) (CAS: Linoleic acid) at 16.08%, 5-ethylundecane at 9.43%, 1,3,5-trimethylbenzene (CAS: Benzene, 1,3,5-trimethyl-) at 8.82%, 1-nonadecene at 7.83%, 6,11-hexadecadien-1-ol at 6.37%, 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS: Bis(2-ethylhexyl) phthalate) at 6.32%, and nonanamide at 5.96%.



**Figure 2:** Chromatogram of crude *J. gossypifolia* seed oil extract

Additional active compounds were also detected in this GC-MS analysis, including possible alkaloids, saponins, tannins, and terpenoids in small amounts. These results align with literature studies,<sup>1</sup> which indicate that the phytochemical content of *J. gossypifolia* varies depending on the plant part used and the type of solvent employed. Seeds collected from Kupang Regency, East Nusa Tenggara, Indonesia, contain a high concentration of linoleic acid, in both raw and roasted forms. This value is nearly identical to the fatty acid content found in *Jatropha curcas*.<sup>21</sup> Long-chain fatty acids and esters are components of plant resins and essential oils extracted from plants. Essential oils are secondary metabolites that are volatile and possess a strong odor. In nature, essential oils play a crucial role in plant protection as antibacterial, antiviral, antifungal, and insecticidal agents, while the release of plant odors reduces herbivore appetite. Additionally, essential oils act as insect attractants that facilitate pollination.<sup>24,25</sup> Linoleic acid, or 9,12-Octadecadienoic acid, is widely found in plant glycosides and serves as an essential fatty acid in mammalian nutrition for the biosynthesis of prostaglandins and cells,<sup>26</sup> antimicrobial,<sup>27</sup> anti-inflammatory.<sup>28</sup>

Different treatments result in varying dissolved chemical components. Raw seeds contain more compounds compared to roasted seeds, likely because the heat during roasting degrades heat-sensitive compounds and simultaneously causes the evaporation of aromatic compounds. This is consistent with the findings of Rohadi et al.,<sup>29</sup> which showed that heating plum java extract reduces the levels of total phenolics, flavonoids, and total tannin content. Phytopolyphenols are a collective term used for plant components with several hydroxyl groups in their molecular structure. Polyphenols, including phenolic acids, flavonoids, lignans, lignins, phlobaphenes, and tannins, are abundant in cereals, vegetable oils, fruits, and vegetables.<sup>30</sup>

Researchers have found that *J. gossypifolia* seeds contain oil with fatty acids as the main components, with heptadecanoic acid being the most commonly found at 35.81%,<sup>13</sup> followed by palmitic and stearic acids<sup>14</sup> which are saturated fatty acids, and oleic and linoleic acids,<sup>15</sup> which are unsaturated fatty acids.<sup>14</sup> Hexadecanoic acid was also reported at 18.05%,<sup>17</sup> along with the presence of methyl esters.<sup>18</sup> These findings are almost identical to those found in the studied extract.

Ethyl 9,12-Octadecadienoate and 9,12-Octadecadienoic acid, or Linoleic Acid, share the same nomenclature, and are the most abundant fatty acid components in each tested sample. This result aligns with the findings of Rosselli et al.,<sup>31</sup> who reported that the oil extract of *Helleborus bocconei* subsp. *intermedius* contains mostly (Z,Z)-9,12-octadecadienoic acid at 70%. However, docosanoic acid and hexadecanoic acid ethyl ester (CAS: ethyl palmitate) were present in higher amounts in this study compared to those found by Rosselli et al.<sup>31</sup>

**Table 1:** Active Compounds in Roasted *J. gossypifolia* Seed Oil Identified by Mass Spectrometer

No.	Compound Name	Retention Time	Formula	Area %
1a	Nonane, 3,7-dimethyl- (CAS) 3,7-Dimethylnonane	28.995	C11 H24	1.25
1b	Pentadecane		C15 H32	
1c	Tetradecane (CAS) n-Tetradecane		C14 H30	
2	Hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate	33.095	C18 H36 O2	7.75
3a	9,12-Hexadecadienoic acid, methyl ester (CAS) METHYL-9,12-Hexadecadienoate	35.125	C17 H30 O2	2.79
	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS) Methyl linolelaidate		C19 H34 O2	
3b	11,14-Eicosadienoic acid, methyl ester (CAS) Methyl-11,14-Eicosadienoate		C21 H38 O2	
4	Ethyl linoleate Linoleic Acid, Ethyl Ester \$\$ Ethyl 9,12-Octadecadienoate	36.386	C20 H36 O2	30.17
5	Ethyl Octadec -9-Enoate	36.469	C20 H38 O2	10.51
6a	Ethyl ester of Docosanoic	36.942	C24H48O2	9.88
6b	Octadecanoic acid, ethyl ester (CAS) Ethyl stearate		C20 H40 O2	
6c	Nonadecanoic acid, ethyl ester		C21 H42 O2	
7a	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS) Glycerol -1,3-Dioctadecanoate	38.825	C39 H76 O5	3.53
7b	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS) Glycerol 1,3-Dihexadecanoate	38.823	C35 H68 O5	3.53
8a	Cyclotetradecane (CAS)	40.367	C14 H28	0.90
8b	Germacrane -B		C15 H30	
bc	5-Octadecene, (E)- (CAS)		C18 H36	
9a	Iron, tricarbonyl[N-(phenyl-2-pyridinylmethylene)benzenamine-N,N']-	40.431	C21 H14 FE N2O3	1.30
9b	Dodecane, 2,6,10-trimethyl- (CAS) Farnesane		C15 H32	0.92
10a	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- (CAS) 2-CIS,CIS-9,12-Octadecadienyloxy	41.012	C20 H38 O2	
10b	1,E-11,Z-13-Octadecatriene		C18 H32	
10c	1,E-8,Z-10-Hexadecatriene		C16 H28	
11a	(Z)6,(Z)9-Pentadecadien-1-ol	41.822	C15 H28 O	19.59
11b	9,17-Octadecadienal, (Z)- (CAS) CIS,CIS-Octadeca -9,17-Dienal		C18 H32 O	
11c	9,12-Octadecadien-1-ol (CAS) Octadeca -9,12-Dien-1-Ol		C18 H34 O	
12a	Heptadecane, 2,6,10,15-tetramethyl- (CAS) 2,6,10,15-Tetramethylheptadecane	42.065	C21 H44	4.05
12b	Octacosane (CAS) n-Octacosane		C28 H58	
12c	Heptacosane (CAS) n-Heptacosane		C27 H56	
13a	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate	43.078	C24 H38 O4	4.14
13b	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate		C24 H38 O4	
13c	1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS) Isooctyl phthalate		C24 H38 O4	
14a	3,3-Dichloro -1,1,2,2-Tetramethyl-Cyclopropane	44.621	C7 H12 CL2	3.21
14b	Silane, [1-(5-ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethoxy]trimethyl-, trans- (CAS)		C13 H26 O2	
14c	1,3-Dioxolane, 4-[(2-methoxy-4-hexadecenyl)oxy]methyl]-2,2-dimethyl- (CAS) 2,3-O-Isopropylidene-1-O-(2-Methoxy 4-He		C23 H44 O4	

**Table 2:** Active Compounds in Crude *J. gossypifolia* Seed Oil Detected by Mass Spectrometer

No.	Compound Name	Retention Time	Formula	Area %
1a	Benzene, 1,3,5-trimethyl- (CAS) 1,3,5-Trimethylbenzene	7.731	C <sub>9</sub> H <sub>12</sub>	8.82
1b	Decane		C <sub>10</sub> H <sub>22</sub>	
2a	Undecane, 5-ethyl-	10.617	C <sub>13</sub> H <sub>28</sub>	2.57
2b	Dodecane		C <sub>12</sub> H <sub>26</sub>	
2c	Undecane		C <sub>11</sub> H <sub>24</sub>	
3a	Benzene, 1,2,3,4-tetramethyl- (CAS) Prehnitol	11.392	C <sub>10</sub> H <sub>14</sub>	1.72
3b	Benzene, 1,2,3,5-tetramethyl- (CAS) 1,2,3,5-Tetramethylbenzene		C <sub>10</sub> H <sub>14</sub>	
4	2-Propenoic acid, 2-ethylhexyl ester (CAS) 2-Ethylhexyl acrylate	14.554	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	2.32
5a	Decane, 6-ethyl-2-methyl-	19.277	C <sub>13</sub> H <sub>28</sub>	0.95
5b	Hexadecane		C <sub>16</sub> H <sub>34</sub>	
5c	Tetradecane		C <sub>14</sub> H <sub>30</sub>	

6	Heptadecane	24.389	C <sub>17</sub> H <sub>36</sub>	1.39
7	Eicosane	28.987	C <sub>20</sub> H <sub>42</sub>	1.68
8a	3-Eicosene	33.035	C <sub>20</sub> H <sub>40</sub>	1.75
8b	1-Nonadecene		C <sub>19</sub> H <sub>38</sub>	
8c	9-Eicosene		C <sub>20</sub> H <sub>40</sub>	
9	Pentadecane (CAS) n-Pentadecane	33.151	C <sub>15</sub> H <sub>32</sub>	1.81
10a	11,14-Eicosadienoic acid, methyl ester (CAS) Methyl-11,14-Eicosadienoate	35.122	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	1
10b	9,12-Hexadecadienoic acid, methyl ester (CAS) METHYL-9,12-Hexadecadienoate HEXADECADIENOATE		C <sub>17</sub> H <sub>30</sub> O <sub>2</sub>	
10c	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS) Methyl linolelaidate		C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	
11a	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid	36.467	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	16.08
11b	6-Pentadecen-1-ol		C <sub>15</sub> H <sub>30</sub> O	
12a	Cyclopropanoic acid, 2-[[2-(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester (CAS)	36.641	C <sub>22</sub> H <sub>38</sub> O <sub>2</sub>	3.35
12b	Ethyl linoleate		C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	
13	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid	36.783	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	9.43
14	1-Nonadecene	36.875	C <sub>19</sub> H <sub>38</sub>	7.83
15a	Heptacosane	36.969	C <sub>27</sub> H <sub>56</sub>	4.15
15b	Pentadecane		C <sub>15</sub> H <sub>32</sub>	
16a	Undecansaeureamid, 11-Bromo	37.036	C <sub>11</sub> H <sub>22</sub> BR N O	5.96
16b	Nonanamide		C <sub>9</sub> H <sub>19</sub> N O	
16c	Hexadecanamide		C <sub>16</sub> H <sub>33</sub> N O	
17a	Cyclohexanol, 2-methyl-3-(1-methylethenyl)-, (1.alpha.,2.alpha.,3.alpha.)- (CAS) CIS,CIS-Isopropenyl -2-Methyl-3-Cyclohexanol	37.200	C <sub>10</sub> H <sub>18</sub> O	1.16
17b	6-Nitro-cyclohexadecane-1,3-dione 1,3-Cyclohexadecanedione, 6-nitro- (		C <sub>16</sub> H <sub>27</sub> N O <sub>4</sub>	
17c	2-Nitro-2-(3'-hydroxybutyl)cyclododecanone 2-(3-hydroxybutyl)-2-nitro-		C <sub>16</sub> H <sub>29</sub> N O <sub>4</sub>	
18a	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS) Glycerol-1,3-Dioctadecanoate 1,3-Distearin	38.827	C <sub>39</sub> H <sub>76</sub> O <sub>5</sub>	1.22
18b	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS) Glycerol 1,3-Dihexadecanoate		C <sub>35</sub> H <sub>68</sub> O <sub>5</sub>	
19a	1-Octadecanol (CAS) Stenol	40.358	C <sub>18</sub> H <sub>38</sub> O	2.61
19b	9-Tricosene,		C <sub>23</sub> H <sub>46</sub>	
20a	Decane, 3,8-dimethyl- (CAS) 3,8-Dimethyldecane	40.437	C <sub>12</sub> H <sub>26</sub>	2.37
20b	Tridecane		C <sub>13</sub> H <sub>28</sub>	
21a	6,11-Hexadecadien-1-ol	41.830	C <sub>16</sub> H <sub>30</sub> O	6.37
21b	(Z)6, (Z)9-Pentadecadien-1-ol		C <sub>15</sub> H <sub>28</sub> O	
21c	9,12-Octadecadien-1-ol (CAS) Octadeca-9,12-Dien-1-ol		C <sub>18</sub> H <sub>34</sub> O	
22a	Heptadecane, 2,6,10,15-tetramethyl- (CAS) 2,6,10,15-Tetramethylheptadecane	42.065	C <sub>21</sub> H <sub>44</sub>	2.33
22b	Hentriacontane		C <sub>31</sub> H <sub>64</sub>	
22c	Tetracosane		C <sub>24</sub> H <sub>50</sub>	
23a	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate	43.086	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	6.32
23b	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate		C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	
24a	Cyclododecane	43.578	C <sub>12</sub> H <sub>24</sub>	1.61
24b	Cyclotetradecane		C <sub>14</sub> H <sub>28</sub>	
26	Ethanol, 2-(9,12-octadecadienyloxy)	44.089	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	1.09
27a	3,3-Dichloro-1,1,2,2-Tetramethyl-Cyclopropane	44.636	C <sub>7</sub> H <sub>12</sub> Cl <sub>2</sub>	2.06
27b	Silane, [1-(5-ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethoxy]trimethyl-, cis- (CAS) Linalool oxide			

These essential oils act as anti-inflammatory and antibacterial agents effective against both Gram-positive and Gram-negative bacteria. The high levels of these essential fatty acids suggest their potential as inhibitors of various bacterial species. Researcher identified n-hexadecanoic acid in the methanol extract of *Eichhornia crassipes* leaves, noting its role in inhibiting both fungal and bacterial growth.<sup>32</sup>

## Conclusion

The GC-MS analysis resulted in 14 active substance peaks from the roasted *J. gossypifolia* seed oil extract, and 27 peaks from the raw *J. gossypifolia* seed oil extract, with variations in bioactive content based on retention time and molecular structure. Among the two extracts, the most abundant components belong to the linoleic acid fatty acid group. Based on this study, it is recommended that further research could be conducted on the antimicrobial properties of the extract for potential

pharmaceutical or veterinary applications. Prospectively, this extract could serve as an alternative drug candidate for treating animal diseases.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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